NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 444

ONE-YEAR INITIATION/PROMOTION STUDY OF *o*-BENZYL-*p*-CHLOROPHENOL

(CAS NO. 120-32-1)

IN SWISS (CD-1®) MICE

(MOUSE SKIN STUDY)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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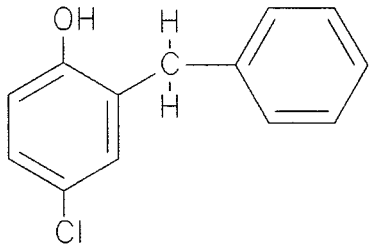
ABSTRACT



o-BENZYL-*p*-CHLOROPHENOL

CAS No. 120-32-1

Chemical Formula: $C_{13}H_{11}ClO$ Molecular Weight: 218.69

Synonyms: 4-Chloro-α-phenol-*o*-cresol; *p*-chloro-*o*-benzylphenol; 2-benzyl-4-chlorophenol; 2-hydroxy-5-chlorodiphenylmethane; 4-chloro-2-(phenylmethyl)phenol; 4-chloro-2-benzylphenol

Trade names: Bio-Clave; Chlorophene; Clorofene; Clorophene; Ketolin H; Nipacide BCPR; Preventol BPR; Santophen 1; Septiphene

o-Benzyl-*p*-chlorophenol (BCP), an aryl halide, is a broad spectrum germicide used in disinfectant solutions and soap formulations in United States hospitals and households. Human exposure to BCP occurs by absorption through the skin and mucous membranes and by ingestion. BCP was studied because of the widespread human exposure and because BCP is an irritant and certain phenolic compounds are weak promoters of skin neoplasia. Groups of Swiss (CD-1®) mice were used to study BCP in a 1-year mouse skin initiation/promotion protocol. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

1-YEAR INITIATION/PROMOTION STUDY

Groups of 50 male and 50 female Swiss (CD-1®) mice were topically exposed to BCP to study its effect as an initiator, promoter, and complete carcinogen. A number of control groups were included in these studies as a reference for the responses of the mouse skin to *o*-benzyl-*p*-chlorophenol (see following table).

Dose Regimen for Reference Controls in the 1-Year Initiation/Promotion Study of o-Benzyl-p-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
Acetone	Acetone	Vehicle Control
Acetone	20 μg DMBA	Reference Complete Carcinogen Control
50 μg DMBA	Acetone	Reference Initiator Control
50 μg DMBA	5 μg TPA	Reference Initiator/Promoter Control
5 μg TPA	5 μg TPA	Reference Promoter Control

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

BCP in acetone was tested as an initiator with the promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The potential of BCP as an initiator was studied by applying a single 100 μL dose of BCP in acetone at a concentration of 10 mg/mL to the dorsal interscapular region of the backs of mice during week 1 of the study. Following the initial BCP application, mice were administered promoting doses of 5 μg TPA three times per week in 100 μL acetone for the first 6 months of the study and once weekly for the final 6 months of the study. BCP in acetone was tested as a promoter with the initiator 7,12-dimethylbenz(a)anthracene (DMBA). Mice were administered a single initiating dose of 50 μL DMBA in 100 μL acetone. Beginning on the second week of the study, mice received 100 μL applications of 0.1, 1.0, or 3.0 mg BCP in acetone three times weekly for up to 51 weeks. Comparative control groups used during the study of BCP as a promoter included: vehicle control (acetone/acetone); promoter control (TPA/TPA); and initiator control (DMBA/acetone). The potential for BCP to act as a complete carcinogen was studied by applying a single initiating dose of 10 mg BCP in 100 μL of acetone, followed by tri-weekly 100 μL applications of 0.1, 1.0, or 3.0 mg BCP to 50 male and 50 female Swiss (CD-1®) mice for 52 weeks. The responses of these groups were compared to vehicle control (acetone/acetone) and complete carcinogen control (acetone/DMBA) groups. The following table shows the various groups with BCP as a promoter, an initiator, and as a complete carcinogen.

Dose Regimen in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
10 mg BCP	0.1 mg BCP	Low-Dose as Complete Carcinogen
10 mg BCP	1.0 mg BCP	Mid-Dose as Complete Carcinogen
10 mg BCP	3.0 mg BCP	High-Dose as Complete Carcinogen
10 mg BCP	5 μg TPA	BCP as Initiator
50 μg DMBA	0.1 mg BCP	BCP Low-Dose as Promoter
50 μg DMBA	1.0 mg BCP	BCP Mid-Dose as Promoter
50 μg DMBA	3.0 mg BCP	BCP High-Dose as Promoter

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

Results in the Study of BCP as a Complete Carcinogen

BCP acted as an irritant when tested as a complete carcinogen using a single initiating dose of 10 mg BCP followed by repetitive applications of 0.1, 1.0, or 3.0 mg BCP for up to 52 weeks, and many of the mice developed cutaneous lesions of scaling/crusts and ulceration. During the course of the study, a single papilloma was first observed after 12 weeks in one 0.1 mg BCP male mouse. One 3.0 mg BCP female was observed with a papilloma at week 10, and three 0.1 mg BCP females were observed with papillomas between weeks 22 and 27. No mice administered BCP/BCP had papillomas at the end of the study, and no malignant cutaneous epithelial tumors were observed at the application sites on any BCP/BCP mice. Thus, in the present study, BCP was not a complete carcinogen.

Results in the Study of BCP as an Initiator

One vehicle control (acetone/acetone) male mouse had developed crusts at the site of application at necropsy, but no male or female vehicle controls had developed papillomas. Mice administered BCP/TPA developed application site lesions including scaling/crusts, ulceration, and irritation; the incidences of these lesions were similar to those in the initiator/promoter control (DMBA/TPA) groups. After 22 weeks papillomas were observed in 12/50 male mice administered BCP/TPA. After 12 weeks papillomas were observed in 7/50 female mice administered BCP/TPA. However, the incidences of papillomas in mice administered BCP/TPA were lower than those in mice administered TPA/TPA (males, 16/50; females, 16/50) and were much lower than those in DMBA/TPA mice (males, 40/50; females, 48/50). Although the incidences of papillomas in mice administered BCP as an initiator were significantly greater than those in the vehicle controls, the incidences were not significantly different from those in TPA/TPA mice. Thus, in the present study, BCP did not demonstrate initiating potential.

Results in the Study of BCP as a Promoter

During the course of the study, incidences of scaling and/or crusts, ulceration, and irritation were observed at the site of application in DMBA/BCP male and female mice, and the incidences were dose related. Incidences of scaling and/or crusts, ulceration, and irritation in 3.0 mg BCP mice were similar to the incidences of these lesions in initiator/promoter control (DMBA/TPA) group, but much higher than the incidences of these lesions in the initiator control (DMBA/acetone) group. A dose-related increased incidence of papillomas was observed in males (DMBA/acetone, 8/50; DMBA/0.1 mg BCP, 3/50;

DMBA/1.0 mg BCP, 5/50; and DMBA/3.0 mg BCP, 14/50) and females (2/50, 6/50, 6/50, and 18/50). The incidence of papillomas in DMBA/3.0 mg BCP females was significantly greater ($P<0.001$) than that in DMBA/acetone females; the incidence of papillomas in DMBA/3.0 mg BCP males was marginally increased ($P=0.077$). No acetone/acetone mice developed papillomas. Although a higher percentage of DMBA/3.0 mg BCP mice developed papillomas over the course of the study than did DMBA/acetone controls, the time it took for half of the number of responding animals to develop papillomas was similar between DMBA/acetone groups and DMBA/3.0 mg BCP groups (DMBA/acetone males, week 38; DMBA/acetone females, week 34; DMBA/3.0 mg BCP males, week 36; DMBA/3.0 mg BCP females, week 37). However, the time to appearance of the first papilloma was shorter in DMBA/3.0 mg BCP mice (males, week 18; females, week 10) than in DMBA/acetone mice (males, week 26; females, week 27). BCP was considered to have promotion potential because the incidences of papillomas in mice treated with DMBA/3.0 mg BCP were greater than those in DMBA/acetone (initiator control) mice and because topical exposure to BCP alone caused no significant increased incidence of papillomas. However, the incidences of papillomas in DMBA/3.0 mg BCP mice (males, 14/50; females, 18/50) were much less than the incidences in DMBA/TPA (promoter control) mice (males, 40/50; females, 48/50); thus, BCP was classified as a weak promoter.

GENETIC TOXICOLOGY

o-Benzyl-*p*-chlorophenol did not induce gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. All tests were performed with and without S9 activation.

CONCLUSIONS

Under the conditions of this 1-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen*.

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* A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on *o*-benzyl-*p*-chlorophenol on November 16, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 16, 1993, the draft Technical Report on the initiation and promotion study of *o*-benzyl-*p*-chlorophenol (BCP) received public review by the National Toxicology Program Board of Scientific Counselors Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. W.C. Eastin, NIEHS, introduced the initiation and promotion study of BCP in Swiss (CD-1®) mice by discussing the properties and uses of the chemical, describing the experimental design, and commenting on the compound-related skin lesions in male and female mice. The proposed conclusions for the study were that in Swiss (CD-1®) mice, BCP was a cutaneous irritant and a weak promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen.

Dr. Ward, a principal reviewer, agreed in principle with the proposed conclusions except that he thought the words "skin tumor" should be added between "weak" and "promoter" in the first sentence of the conclusions. He suggested that the weak promoting activity of BCP may be due to skin wounding (irritation) rather than the effects of BCP on skin carcinogenesis/promotion, and provided copies of journal articles describing such effects. Dr. Eastin said he was familiar with the papers and would add information on wounding and skin irritation as well as discussion on other weak promoters.

Dr. Taylor, the second principal reviewer, said the proposed conclusion that BCP was a weak promoter was overstated considering that DMBA/acetone groups of mice developed many, if not more neoplasms than did the DMBA/BCP groups. Further, the inconclusiveness of the findings was compounded by the degree of irritation and skin wounding that occurred. Dr. Taylor thought that more discussion was needed.

Dr. Ryan, the third principal reviewer, commented that although the proposed conclusions were in line with the findings, she was concerned with the ambiguity of some of the results, such as the fact that higher incidences of some lesions were seen with DMBA/acetone than with DMBA/BCP. In response to Drs. Taylor and Ryan, Dr. Eastin said that the incidences of skin papillomas were clearly elevated in the high dose DMBA/BCP group relative to DMBA/acetone. The designation of "weak promoter" derived from a comparison of DMBA/BCP with the DMBA/TPA reference controls with TPA representing a strong or potent promoter. Dr. Ryan wondered how to interpret the results of this study in light of the gavage study and asked whether there had been studies of absorption of BCP following dermal administration. Dr. Eastin said that NIEHS studies have shown that BCP is well absorbed after dermal administration.

Dr. Ward moved that the Technical Report on *o*-benzyl-*p*-chlorophenol be accepted with the revisions discussed and the conclusions as written with the addition of "skin tumor" to read as follows: Under the conditions of this one-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen. Dr. Taylor seconded the motion, which was accepted unanimously with five votes.